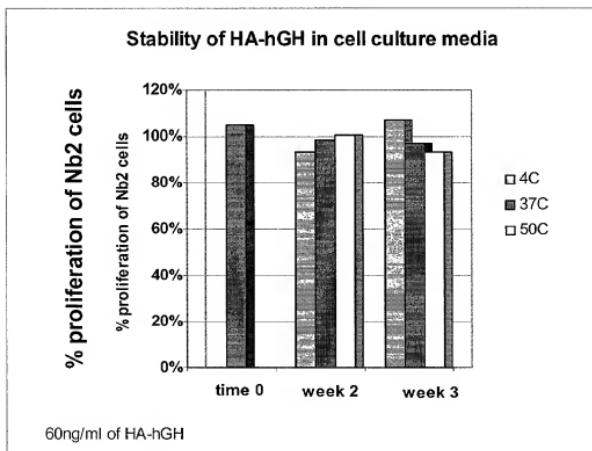
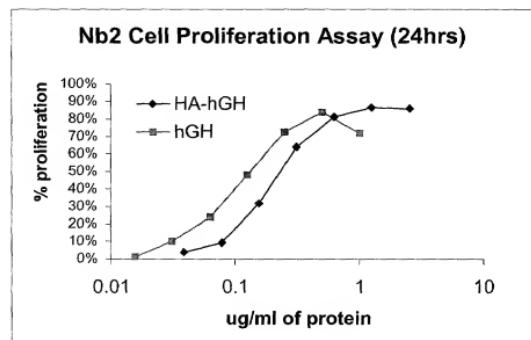


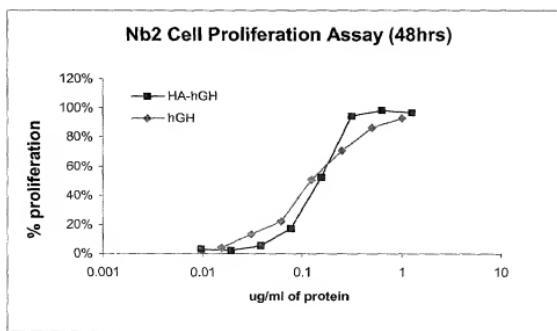
**Figure 1**



**Figure 2**



**Figure 3A**



**Figure 3B**

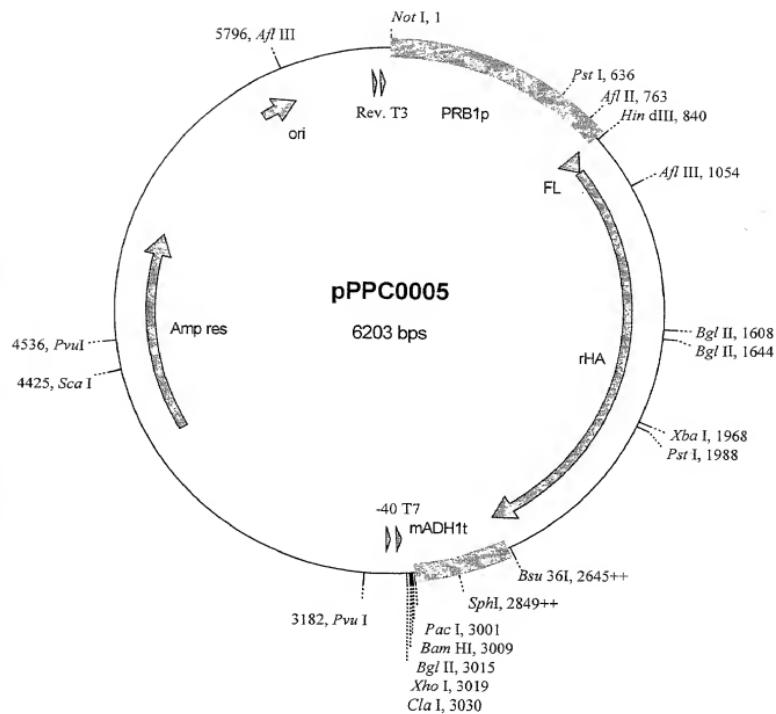
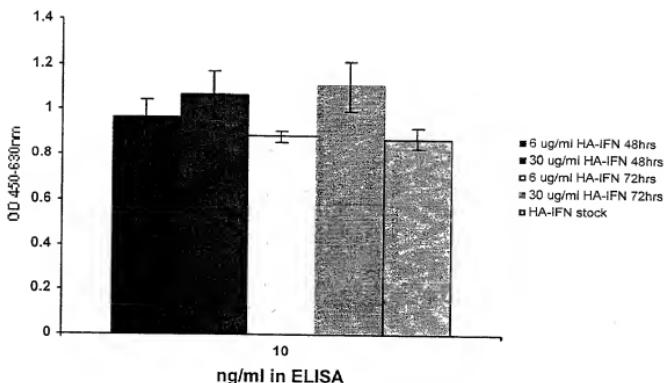


Figure 4



**Figure 5**

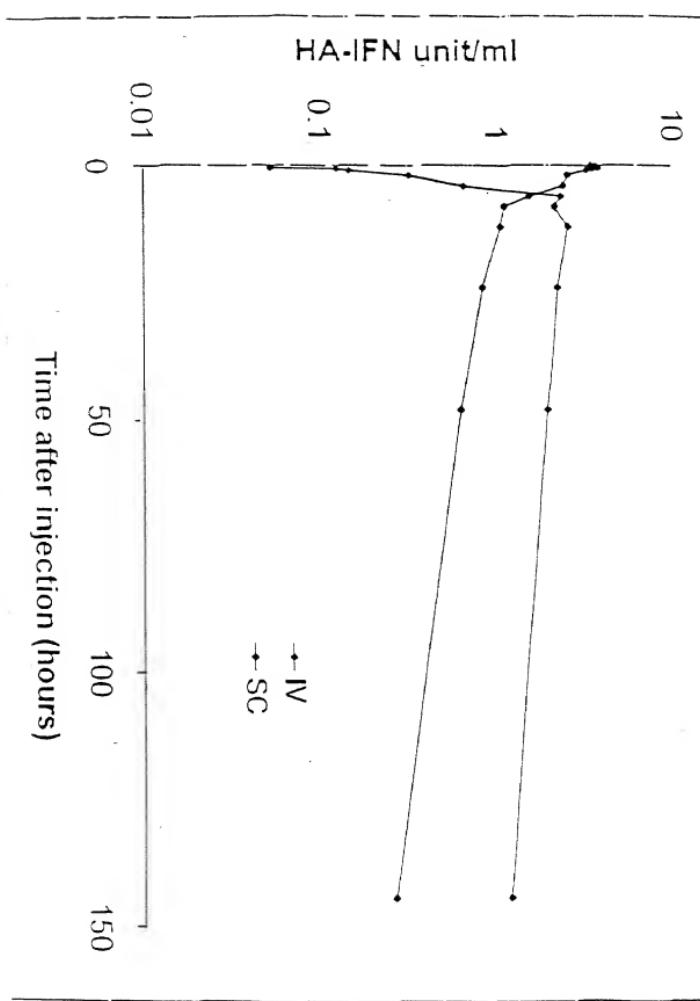
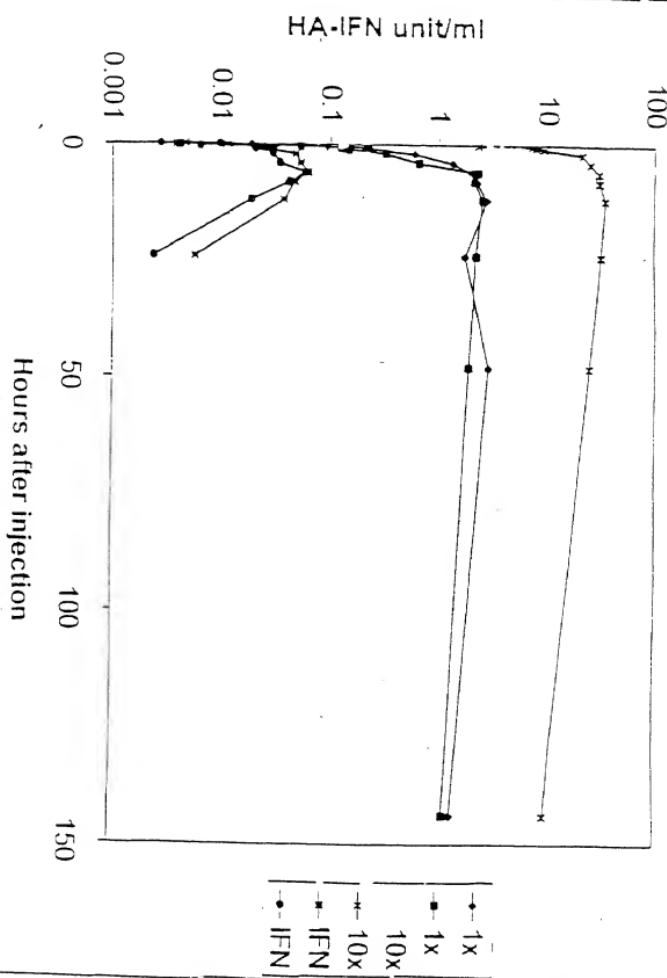
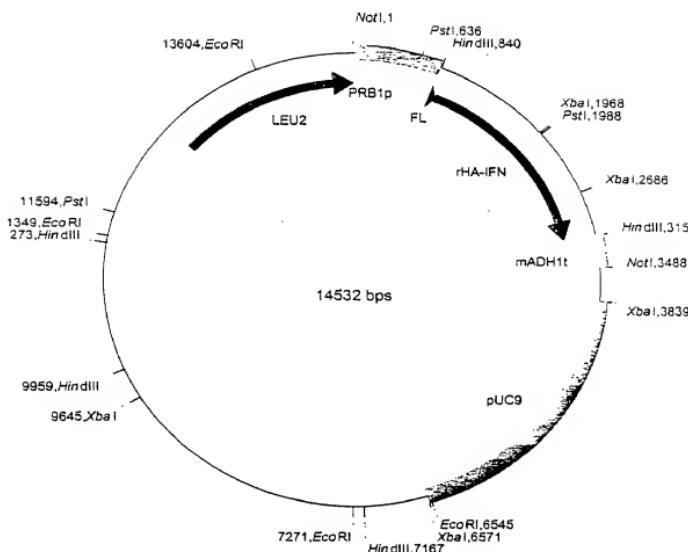


Figure 6





**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises  
*PRB1* promoter, from *S. cerevisiae*.  
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF $\alpha$ -1 leader.  
 HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)  
*ADH1* terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam*HI fragment generally used.

**Figure 8**

## **Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion**

## Loop

I Val54-Asn61  
II Thr76-Asp89  
III Ala92-Glu10  
IV Gln170-Ala1  
V His247-Glu2  
VI Glu266-Glu2

## Loop

VII Glu280-His288  
 VIII Ala362-Glu368  
 IX Lys439-Pro447  
 X Val462-Lys475  
 XI Thr478-Pro486  
 XII Lys560-Thr566

**Figure 9**

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHHH HHHHHHHHHH HHHHHHHHHHHH HHHHHHHHHHHH

IV

151 APELLFFAKR YKAATTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHH HHHHHHHHHH HHHHHHHHHHHH HHHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

**b. Insertion (or replacement) of Randomised sequence into Loop IV.**

(X)<sub>n</sub>  
 ↓  
 IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHH HHHHHHHHHH HHHHHHHHHHHH HHHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

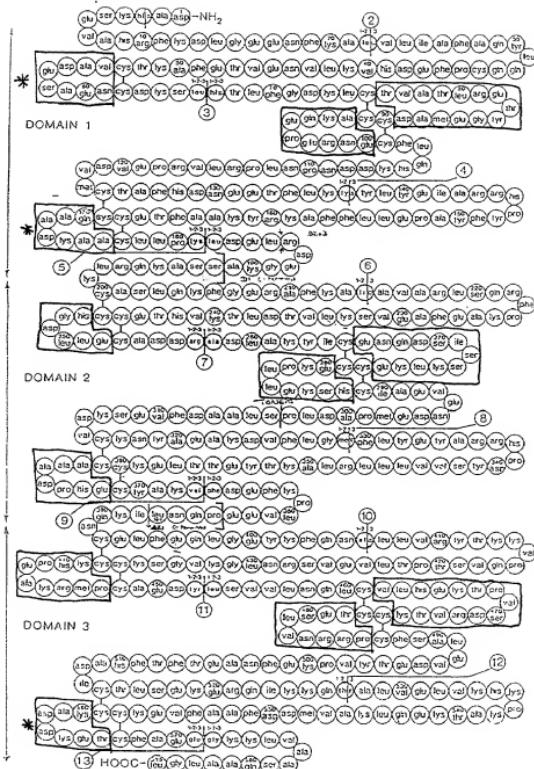
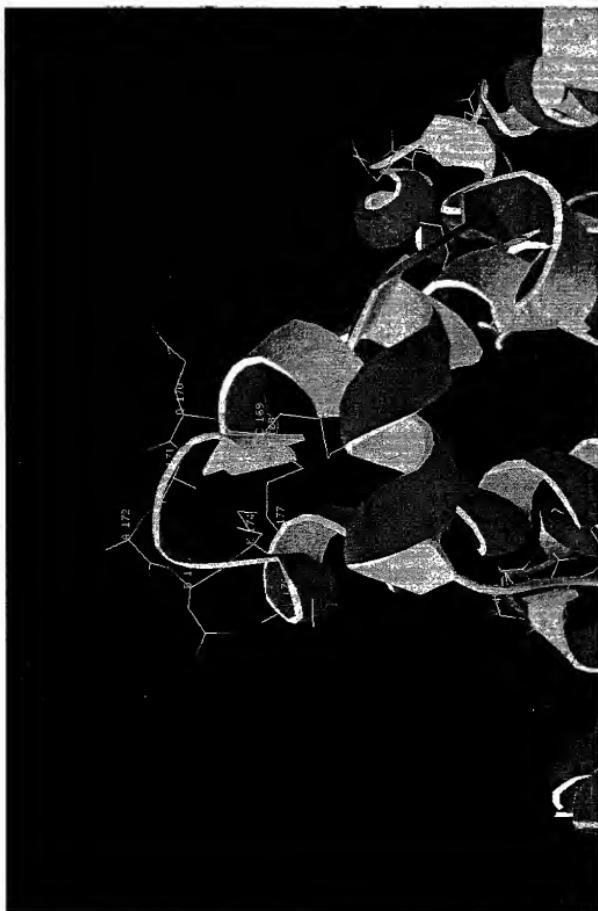


Figure 11



Disulfide bonds shown in yellow

**Figure 12: Loop IV Gln170-Ala176**

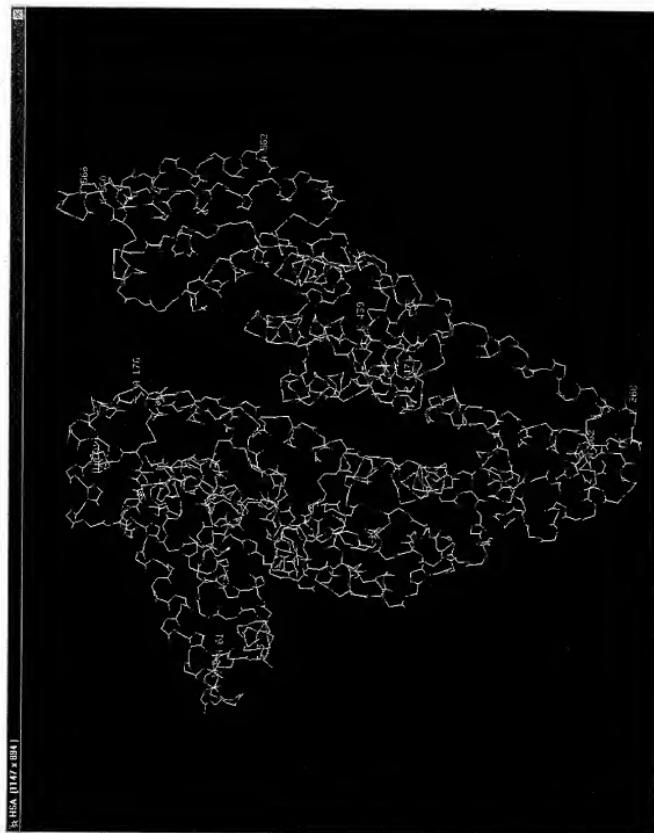
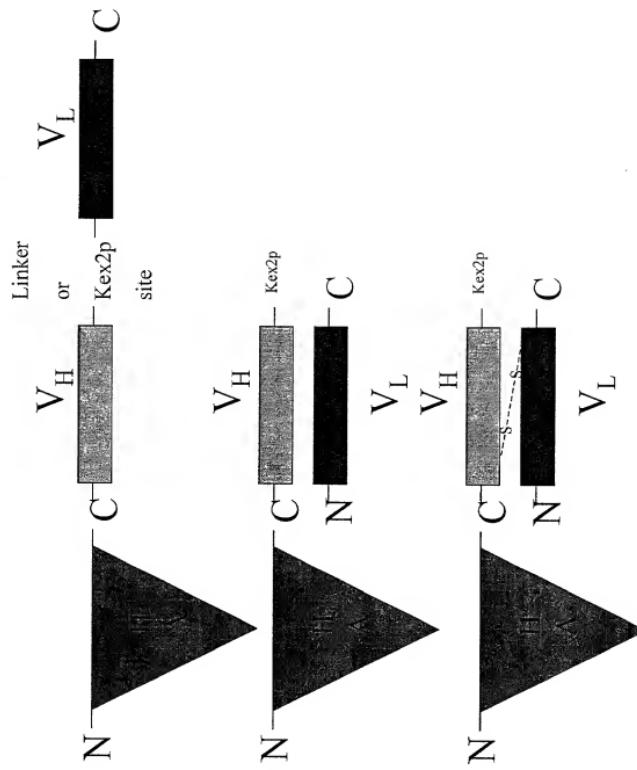


Figure 13: Tertiary Structure of HA



**Figure 14: Schematic Diagram of Possible ScFv Fusions  
(Example is of a C-terminal fusion to HA)**

## 15/18

1 GAT GCA CAC ANG AGT GAG CGG GTC GCT GAT CGT CGG TTT AAA GAT TTT TGC GGA GAA GAA AAT TTC AAA 60  
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTC TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAA GAT CAT GTA 120  
21 A L V L I A P A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTC ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180  
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GTC AAA TCA CTT GAT AAC CTT TTT GCA GTC AAA TTA TGC ACA GTC GAA ACT CCT 240  
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TUG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TUG GTG AGA CCA GAG GTC 360  
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTC ATG TGC ACT GCT GTC ATT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CGG GAA CTC CTT TTC TTT GCT AAA AGG 480  
141 E I A R R H P Y F Y A P E L L F F A K R 160

Figure 15A

## 16/18

481 TAT AAA GCT GCT TTT AGA GAA TGT TGC CAA GCT GAT AAA GCT GAT AAA D K A A D K A A C L L P 180  
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GGC AAA CAG AGA CTC AAA TGT 600  
191 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTC AGC 660  
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720  
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTC 780  
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840  
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900  
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TGT CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTC AAA AAC TAT GCT 960  
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

## 17/18

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TGT TAT GCA AGA AGG CAT CCT GAT 1020  
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080  
341 Y S V V L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CCT 1140  
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200  
381 V E P Q N L I K Q N C B L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260  
401 Y K F Q N A L L V R Y T K K V P Q V S T T 420

1261 CCA ACT CTT GTC GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320  
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CGT AAC CAG TTA 1380  
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTC AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440  
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

## 18/18

1441 TTG GTG AAC AGG CGA CGA TGC TTT TCA GCT CTT GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500  
481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TCC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560  
501 E P N A E T F T P H A D I C T L S S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTT GAG AAA CAC AAG CCC AAG GCA ACA 1620  
521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTC GAG AAG TGC TGC AAG 1680  
541 K E Q L K A V M D D P A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740  
561 A D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCG TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782  
581 A A L G L \* 585

Figure 15D